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THE UNIVERSITY OF ALBERTA

THE EVOLUTIONARY RELATIONSHIP AMONG COLONIES OF COLUMBIAN GROUND SQUIRRELS (RODENTIA: SCIURIDAE)

by

DONNA M. MAC NEIL

(C)

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

IN

ZOOLOGY

EDMONTON, ALBERTA

SPRING 1986

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled THE EVOLUTIONARY RELATIONSHIP AMONG COLONIES OF COLUMBIAN GROUND SQUIRRELS (RODENTIA: SCIURIDAE) submitted by DONNA M. MAC NEIL in partial fulfilment of the requirements for the degree of MASTER OF SCIENCE in ZOOLOGY.

Supervisor

Date February 5, 1986

Abstract

Mitochondrial DNA (mtDNA) obtained from 71 Columbian ground squirrels (*Spermophilus columbianus*) collected in 12 locations throughout their range in western Canada were digested with 10 restriction endonucleases to infer the history of colonization by this species of its extant distribution in Canada. Five of these endonucleases revealed variant patterns in populations from which composite mitochondrial genotypes (clones) were compiled for each individual. The matriarchal transformation series among the mtDNA clones consisted of four clones in a linear array. Two of the clones had a wide distribution within the range, and only one of the 12 colonies was polymorphic for two closely related clones, indicating Columbian ground squirrel populations are very homogeneous with respect to mtDNA sequences. The degree of relatedness of Columbian ground squirrels to two other species of Sciuridae was estimated from comparison with the mtDNA restriction patterns of nine Richardson's ground squirrels (*S. richardsonii*) and two Arctic ground squirrels (*S. parryii*). Calculation of divergences from fragment length and restriction site data indicated that Arctic ground squirrels and Richardson's ground squirrels were more closely related to each other than either were to Columbian ground squirrels. The transformation series among clones within the Columbian ground squirrels was rooted using Richardson's and Arctic ground squirrels as outgroups. From this data, the historical path of dispersal by female founders of Columbian ground squirrel populations was inferred to be from the northern part of the species' distribution southward along the eastern and western ranges of the Rocky Mountains.

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Table of Contents

Chapter — Page

I. Introduction 1

II. Materials and Methods 3

III. Results 5

A. Identification of clones 5

B. The ancestral clone for S. columbianus 6

C. Reconstructed transformation series 8

IV. Discussion 11

A. Diversity of clones 11

B. Pattern of diversity 12

C. Geographical history of S. columbianus 14

Literature cited 16

Appendix A: Materials and Methods 40

A. Field Procedures 40

Collection of Columbian ground squirrels 40

Collection of Richardsons ground squirrels and Arctic ground squirrels 41

B. Laboratory Procedures 41

Method for isolation of mitochondrial DNA from liver tissue 41

Restriction analysis 43

Construction of Restriction Maps 44

C. Restriction Patterns 45

Literature Cited 46

List of Tables

Table	Page
1. Collection sites of Columbian ground squirrels trapped throughout Alberta and British Columbia	20
2. Distribution of mtDNA clones among Columbian ground squirrels in Alberta and British Columbia	21
3. Number of restriction sites shared among Columbian, Arctic, and Richardson's ground squirrels	22
4. Maximum likelihood estimates of divergences (π) among Columbian, Arctic, and Richardson's ground squirrels as calculated from Nei and Tajima (1983)	23
5. Number of fragments shared among Columbian, Arctic, and Richardson's ground squirrels	24
6. Average estimates of divergences (δ) among Columbian, Arctic, and Richardson's ground squirrels as calculated from Nei and Li (1979)	25
A1. Solutions required for isolation and digestion of mtDNA	47
A2. Digest and electrophoresis conditions	48
A3. Fragment lengths derived from restrictions with each of ten endonucleases for the three species of ground squirrels	49

List of Figures

Figure		Page
1.	Locations of collection sites of Columbian ground squirrels in Alberta and British Columbia	27
2.	Restriction patterns of the endonucleases, Hae III, Mbo I, Cfo I, and Eco RI, in Columbian ground squirrels	29
3.	Restriction maps and transformation series of the three species of ground squirrels for Bcl I, Bgl II, Cfo I, Eco RI, Hind III, and Xba I	31
4.	Alternative hypotheses for the character state of presence or absence of the Eco RI restriction site at 6.01 kb	35
5.	Transformation series of Columbian, Arctic, and Richardson's ground squirrel based on all mapped restriction sites	37
6.	Hypothetical dispersal routes of Columbian ground squirrels following the retreat of the Wisconcinan ice age	39

I. Introducton

One of the most important factors influencing the present pattern of variation in natural populations, is the history of colonization by a species of its extant distribution. Thus the pattern of variation within a species' range can be employed to infer the pattern of its historical colonization. Variation in mitochondrial DNA (mtDNA) is especially suitable for this inference because it is predominantly maternally inherited (Dawid and Blacker 1972; Giles *et al.* 1980), making possible the construction of matriarchal lineages unhampered by complications arising from recombination and assortment which plague attempts based on variation in nuclear DNA (Lansman *et al.* 1981). In addition mtDNA evolves ten times more rapidly than nuclear DNA (0.02 sub/bp/10^6 yrs) (Brown *et al.* 1979), and therefore lineages based on mtDNA will diverge more quickly, making it a more sensitive method of differentiating conspecific populations.

Variation in mtDNA can be determined by restriction analysis in which type II restriction endonucleases are used to digest DNA at specific short palindromic sequences. This method has been widely used in population studies to describe variation in and measure divergence of mtDNA sequences among closely related groups of animals. By comparing the presence or absence of restriction sites between mtDNA sequences, it is possible to measure divergence among cogeneric species (Ferris *et al.* 1983, Ferris *et al.* 1981, Avise *et al.* 1979, Templeton 1983) and conspecific populations (Hale and Beckenbach 1985, Kessler and Avise 1985, Johnson *et al.* 1983, Lansman *et al.* 1983).

This study describes the divergence and infers the historical dispersal of the terrestrial rodents, Columbian ground squirrels (*Spermophilus columbianus*) in western Canada. Columbian ground squirrels are distributed along the foothills of the Rocky Mountains from 200 to 2500 m in elevation (Banfield 1974), from eastern British Columbia and western Alberta (ca. 55°N) south into eastern Washington state,

northern Idaho and Oregon, and western Montana (Hall 1981). Since this species currently occupies areas in British Columbia and Alberta which were largely under ice cover during the Wisconcinan ice age, these areas would have been colonized after the ice retreated ca. 12,000 years before present (yrs B.P.).

Columbian ground squirrels are an ideal system in which to use mtDNA because they are organized into colonies from which males disperse more frequently and farther than females (Boag and Murie 1981), as occurs in most species of this genus (Holekamp 1984; Greenwood, 1980), and thus mtDNA lineages will reflect past dispersal patterns. For example, in deer mice (*Peromyscus maniculatus*), divergence in mtDNA among populations increases with geographical distance among populations (Lansman *et al.* 1983), as would be expected from the sexually asymmetrical dispersal of this species (Stickel 1968, cited in Lansman *et al.* 1983). Conversly, restriction analysis of mtDNA has also been used to infer sexually asymmetrical dispersal. Davis and Patton (1985) compared nuclear and mitochondrial markers among populations of pocket gophers (*Geomys bursarius* and *G. attawateri*) and concluded that in these species, males are more likely to disperse than females.

In addition, mtDNA from individuals of two cogeneric species, Richardson's ground squirrels (*Spermophilus richardsonii*) and Arctic ground squirrels (*Spermophilus parryii*) was examined to infer the ancestral type in the matriarchal transformation series of mtDNA of *S. columbianus*, and estimate the degree of divergence among the three species of ground squirrels.

II. Materials and Methods

Seventy-one Columbian ground squirrels were collected from 6 locations in Alberta and 6 locations in British Columbia, which roughly formed two parallel north-south transects through their range in Canada (Fig. 1 and Table 1). Also obtained were nine Richardson's ground squirrels (*S. richardsonii*) from Edmonton, Alberta, and two Arctic ground squirrels (*S. parryii*) from Chilkat Pass, British Columbia. All individuals were trapped and transported live to the Univ. of Alberta, Edmonton, and kept in facilities there until used for extraction of mtDNA. Mitochondria were isolated from liver tissue using the method of Pedersen *et al.* (1978). DNA was extracted from the mitochondria by a method modified after Wills *et al.*(1984); dodecyl sodium sulfate was added to a final concentration of 2% rather than 1% and the lysate was incubated at 37°C for 12 hrs. All samples of mtDNA of Columbian ground squirrels were digested with ten endonucleases according to the manufacturers specifications (Bethesda Research Labs.), and electrophoresed on agarose gels (Maniatis *et al.* 1982). The ten endonucleases with the nucleotide sequence which they recognise in parentheses are Bcl I (TGATCA), Bgl II (AGATCT), Cfo I (GCGC), Dde I (CTNAG), Eco RI (GAATTC), Hae III (GGCC), Hind III (AAGCTT), Hpa II and/or Msp I (CCGG), Mbo I (GATC), and Xba I (TCTAGA). MtDNA from Richardson's and Arctic ground squirrels were digested with all endonucleases above except for Dde I, because of limited samples. Restriction patterns were identified by staining with ethidium bromide and photographing under ultraviolet light.

For each endonuclease that revealed any variation within *S. columbianus*, one restriction pattern was arbitrarily designated A, and the other pattern, B (Fig. 2). The patterns of the five endonucleases that showed variation were compiled into a composite mtDNA genotype (Table 2). Each letter in the series of five represents the restriction pattern derived from the digestion with a particular endonuclease in

order; Cfo I, Dde I, Eco RI, Hae III, and Mbo I. To construct a restriction map for the three species of ground squirrels, double and partial digests of mtDNA of several individual samples were performed. The sites of the endonucleases, Dde I, Hae III, and Mbo I, could not be mapped by the methods used here since the large number of small fragments produced by restriction with any of these endonucleases made interpretation of partial and double restrictions unreliable.

Two methods for the calculation of divergence among the three species were employed. The first method uses mapped restriction sites of tetrameric endonucleases (those that recognize sequences of 4 base pairs (bp) long) and hexameric endonucleases (those which recognize 6 bp) to calculate a single maximum likelihood estimate of divergence (π) (equation 28 in Nei and Tajima 1983). Data from the mapped sites of the endonucleases Bcl I, Bgl II, Cfo I, Eco RI, Hind III, and Xba I, were used to calculate divergence with this method (Table 4). The second method uses lengths of restriction fragments only from digestions of tetrameric endonucleases, or pentameric endonucleases (those which recognize 5 bp), or only hexameric endonucleases to calculate estimates of divergence (δ) (equation 8 in Nei and Li 1979). Data from the tetrameric endonucleases, Cfo I, Hae III, Mbo I, and Msp I, were used to calculate δ, and data from the hexameric endonucleases, Bgl II, Bcl I, Eco RI, Hind III, and Xba I, were used to calculate a second δ. The arithmetic average of the two estimates was taken for a single estimate of divergence (Table 6). The second method has one advantage over the first in that information from endonucleases whose sites have not been mapped can be incorporated into an estimate of divergence.

III. Results

A. Identification of clones

Only five endonucleases, Cfo I, Dde I, Eco RI, Hae III, and Msp I, of the ten used in the survey revealed variation within Columbian ground squirrels in Canada (Fig. 2). (Descriptions of restriction patterns are in Table A3 of the attached appendix.) The A restriction pattern of Cfo I differs from the B pattern by the gain of one restriction site; the A pattern of Eco RI differs from the B pattern by the gain of one restriction site and the loss of another. The sites for these two endonucleases have been mapped with respect to each other and the invariant Bgl II restriction sites (Fig. 3). Because restriction maps of Dde I, Hae III, and Mbo I were not constructed, only the number of fragments shared by the two patterns for each of these endonuclease were compared. Minimal estimates of the number of shared fragments for these endonucleases are: the two restriction patterns of Dde I share 13 fragments, the two patterns of Hae III share 15 fragments, and the two patterns of Mbo I share 14. The number of shared fragments are minimal estimates because the detection and scoring of fragments less than 500 bp in length are difficult with the ethidium bromide staining technique.

Bcl I was the only endonuclease to reveal variation within the sample of Richardson's ground squirrels. The A restriction pattern differs from the B pattern by the loss of one restriction site. No variation was observed within the sample of Arctic ground squirrels.

The types of variant restriction patterns of each endonuclease were compiled into a composite genotype for each individual. For the 71 individuals analysed only four different composite genotypes (clones) were observed. Eleven of the twelve colonies were monomorphic for one of the clones. In only one colony, located near Bluet, B.C., were two clones detected, one of which was unique to that colony

(Table 2). Two of the clones have a wide geographical distribution (Fig. 1): **ABBBB** is found in most of the western locations as well as in two northeastern colonies, and **AAAAA** occurs in the southern Alberta and extends into eastern British Columbia. The remaining two clones were observed in only one colony each and so are limited in their distribution. Among the eastern colonies, three clones are observed, whereas the western colonies are identical to each other except for clone **BBBBB** also present in a colony in southern British Columbia. Three of the clones differ in only one of the five restriction patterns (**ABBBB** differs from **AABBB** in their Dde I restriction patterns and differs from **BBBBB** in their Cfo I patterns). The most divergent pair of clones, **AAAAA** and **BBBBB**, differ in five of their restriction patterns. Both clones are in the central portion of the range of Columbian ground squirrels close to the Canada - United States border but are topographically separated by the Nelson and Moyie Ranges.

The matriarchal transformation series of the four clones of mtDNA is an unrooted linear array: **AAAAA←→AABBB←→ABBBB←→BBBBB**. This linear transformation series can be superimposed on the geographic origins of the samples (Fig. 1) and the distribution of the clones in relation to each other is also linear.

B. The ancestral clone for S. columbianus

In order to identify the ancestral clone of the Columbian ground squirrel variants, two outgroups were examined: Richardson's ground squirrel and Arctic ground squirrel. The range of Richardson's ground squirrels extends from southeastern Alberta, southern Saskatchewan and Manitoba, south into Montana, eastern Idaho and North and South Dakota, overlapping with the range of Columbian ground squirrels in areas of Alberta and Montana. The Arctic ground squirrel is a holarctic species which extends from the Yukon and North West Territories into northern British Columbia to ca. 54°N and does not overlap with

the range of Columbian ground squirrels (Hall 1981). The phyletic relationship among the three sciurid species is uncertain. Electrophoretic analysis of enzymes representing 13 loci (Nadler *et al.* 1982), and multivariate statistical analysis of 36 cranial features (Robinson and Hoffmann 1975) suggest that Columbian ground squirrels are more closely related to Richardson's ground squirrels than to Arctic ground squirrels. Karyotypic analysis has led to different conclusions in different studies: Lyapunova and Vorontsov (1970) proposed that Arctic ground squirrels and Richardson's ground squirrels are more closely related, whereas Nadler *et al.* (1984) proposed that Columbian ground squirrels and Arctic ground squirrels are more closely related. Both were examined to determine the ancestral clone within the matriarchal transformation series of mtDNA of Columbian ground squirrels and to attempt to support one of the proposed phyletic relationships among the three species by restriction analysis of mtDNA.

A network was constructed for the three species from the mapped restriction sites of Bgl II, Bcl I, Cfo I, Eco RI, Hind III, and Xba I. The variant and invariant restriction sites of the endonucleases were mapped for the three species with respect to the invariant Bgl II restriction sites (Fig.3). The number of shared restriction sites among the three species for each of the 6 endonucleases can be counted and summed for the tetrameric endonuclease (Cfo I) and the hexameric endonucleases (Bgl II, Bcl I, Eco RI, Hind III, and Xba I) (Table 3). The number of sites shared in the pairwise comparisons of species are similar, ranging from 21 to 26 total sites shared. Divergences calculated from restriction sites by the method of Nei and Tajima (1983) (Table 4) indicate that Arctic ground squirrels and Richardson's ground squirrels are less divergent from each other (π = 2.18%) than either are from any clone of Columbian ground squirrels. Clones **AABBB** and **ABBBB** are more similar to Arctic ground squirrels (π = 5.26%) than to Richardson's ground squirrels (π = 5.75%). The number of shared fragments

among the three species for Bcl I, Bgl II, Cfo I, Eco RI, Hae III, Hind III, Mbo I, Msp I, and Xba I can also be counted and summed for the tetrameric endonucleases (Cfo I, Hae III, Mbo I, and Msp I) and the hexameric endonucleases (Table 5). The divergence calculated by the method of Nei and Li (1979) among the three species is smallest between Arctic ground squirrels and Richardson's ground squirrels (δ = 2.86%) (Table 6). Clones **AABBB** and **ABBBB** of Columbian ground squirrels are less divergent from Arctic ground squirrels (δ = 6.65%) than from Richardson's ground squirrels (δ = 7.34%). **AABBB** and **ABBBB** are distinguishable only by their Dde I restriction pattern and since mtDNA of Arctic ground squirrels could not be digested with Dde I, the relatedness of these clones to Arctic ground squirrels cannot be more clearly resolved.

C. Reconstructed transformation series

The mtDNA restriction maps for each endonuclease whose sites were mapped with respect to invariant Bgl II sites were arranged into a transformation series (Fig. 3). Bgl II sites are identical among the three species and so give no information about the relatedness of any pair of species. The transformation series among the Hind III restriciton maps requires the postulation of a hypothetical clone to minimize the number of site changes. This hypothetical clone may exist or have existed at some time during the evolution of the Hind III clones. The transformation series among the Bgl I, Cfo I, and Xba I restriction maps are linear, depicting no convergence or parallel loss or gain of any sites.

The evolution of Eco RI sites requires either the convergent loss or gain of one Eco RI site at 6.01 kb in two lineages. Since the divergences as calculated by two methods indicated that Arctic ground squirrels and Richardson's ground squirrels are more closely related to each other than either are to Columbian ground squirrels (Tables 4 and 6), the most probable phylogeny of the three species is that depicted

in Figure 4. Figure 4 depicts two alternatives that differ only in which character state, *i.e.* the presence or absence of 6.01 kb site, is ancestral. In Figure 4a, in order for both Eco RI variant **B** of Columbian ground squirrels and Richardson's ground squirrels to not possess the Eco Ri site, the character must have been lost independently in these lineages. In Figure 4b, in order for both Columbian Eco RI variant **A** and Arctic ground squirrels to have the Eco RI site, the character must have been gained independently in the two lineages. There are 18 ways in which to lose a restriction site of 6 bp, *i.e.* by the substitution of any one of the other three bases for each of the 6 bp in the restriction site. Alternatively, there is only one way in which to gain a restriction site if a sequence of DNA is one base pair away from the restriction site sequence, *i.e.* by the substitution of the "correct" base for the "incorrect" base. Thus it is more probable that the same restriction site is lost twice than the same site is gained twice, *i.e.* the phylogeny in Figure 4a is the more probable of the two and the transformation series among the Eco RI maps depicts the convergent loss of the Eco RI site at 6.01 kb.

A Wagner network of the three species constructed on the basis of parsimony from the mapped restriction sites that were lost and gained, requires 24 character state changes (the loss or gain of a restriction site) (Fig. 5). Arctic ground squirrels and Richardson's ground squirrels diverge from a hypothetical intermediate which is separated from Columbian ground squirrel clones **AABBB** and **ABBBB** by 13 site changes. Arctic ground squirrels differ from the hypothetical intermediate by the loss of the Hind III site at 4.07 kilobase pairs (kb) and the gain of the Xba I sites at 4.08 kb and 5.12 kb. Clone A of Richardson's ground squirrels differs from the hypothetical intermediate by the loss of the Eco RI site at 6.01 kb and the Bcl I site at 1.12 kb and the gain of the Eco RI site at 2.35 kb and the Hind III site at 11.30 kb. Clone **B** of Richardson's ground squirrels differs from clone **A** by the gain of the Bcl I site at 6.80 kb. Within Columbian

ground squirrels, clone **AAAAA** differs from **ABBBB** and **AABBB** at the least by the loss of the Eco RI site at 12.63 kb and the gain of the Eco RI site at 6.01 kb. Clone **BBBBB** differs from **ABBBB** and **AABBB** by the gain of the Cfo I restriction site at 16.05 kb. In this most parsimoneous transformation series, clones **AABBB** and **ABBBB** are ancestral and the convergent loss of one Eco RI site at 6.01 kb is required. An alternative transformation series, in which clone **AAAAA** is ancestral, also requires 24 character state changes, but requires the convergent gain of the Eco RI site at 6.01 kb. A third transformation series, in which **BBBBB** is ancestral, requires 25 character state changes.

IV. Discussion

Two major results of this study, the amount of divergence and the pattern of diversity within populations of Columbian ground squirrels, merit discussion. From the interpretation of these results and with knowledge of the geological history of the area in Canada currently occupied by this species, the pattern of distribution of variation in mtDNA can be used to infer the historical pattern of migration. I conclude that Columbian ground squirrels have been present on the eastern ranges of the Rocky Mountains for at least 100,000 yrs B.P., surviving the Wisconsinan ice age in refugia, and have more recently colonized the western ranges of the Rocky Mountains from the northern part of the current distribution of the Columbian ground squirrels.

A. Diversity of clones

One of the more suprising results in this study was the amount of diversity among the populations of Columbian ground squirrels over their range in Canada. MtDNA restriction analysis with 10 endonucleases revealed only four closely related clones. This number of variants is low when compared to other surveys of mtDNA variation in natural populations. When making comparisons of this nature it is important to remember that the number of clones observed is influenced by the size of the area sampled since populations separated by greater geographical distances are expected to be more divergent. Therefore, selecting from other studies areas of comparable size to that sampled in this study (ca. 15 X 10^6 ha): within Florida, Alabama, and Georgia, Avise *et al.* (1979) observed 23 clones in pocket gophers (*Geomys pinetis*) with 6 endonucleases; within California, Lansman *et al.* (1983) observed 15 in deer mice with 8 endonucleases; within Botswana, Johnson *et al.* (1983) observed 9 clones in humans with 5 endonucleases; and within New York State, Brown and Simpson (1981) observed 8 clones in Norwegian rats (*Rattus*

norvegicus) with 7 endonucleases.

On another level of comparison, the occurence of polymorphic populations in this survey is unusually rare. In all of the above studies (with the possible exception of Johnson *et al.* (1983)) polymorphic populations were observed with greater frequency. Avise *et al.* (1979) observed 5 polymorphic populations of pocket gophers from 26 locations sampled; Lansman *et al.* (1983) observed 17 polymorphic populations of deer mice from 35 locations sampled; and Kessler and Avise (1985) observed 3 clones in one population of cotton rats (*Sigmodon hispidus*) by sampling 134 individuals within a relatively small area (3.2 ha). In this study more than one clone was observed in only one colony and no variation was observed in the largest sample (19 individuals) taken from a colony near Longview, Alta., which covered an area of approximately 2 ha (Waterman 1985). Therefore, compared to other studies, the Columbian ground squirrel populations occupying the sampled area are relatively depauperate of variation in sequence of mtDNA.

B. Pattern of diversity

The first notable feature of the pattern of distribution of the clones is the unequal distribution of diversity between the eastern and western ranges of the Rocky Mountains. On the eastern ranges in the northern colonies, there are two relatively similar clones, **AABBB** and **ABBBB**, and a third clone in the south, **AAAAA**, which is relatively divergent from the northern clones. This is in contrast to the distribution of the clone **ABBBB** among all the western colonies. The greater amount of divergence among the eastern colonies implies an older origin for these colonies than for those of the western ranges.

Since clone **ABBBB** is present in the same colony as clone **BBBBB** and all other colonies along the western ranges are identical, an estimate of time of divergence among these colonies is meaningless. However, an estimate of time of

divergence among the colonies on the eastern ranges is valid. Estimated times of divergences are calculated as $t = \delta/2\lambda$ (from Nei and Li 1979), where δ = estimated divergence, and λ = rate of evolution (ca. 0.02 sub/bp/10^6 yrs). The estimated times of divergences are also calculated by using the relationship between π and δ as $\pi = \frac{3}{4}[1 - e^{-(4\delta/3)}]$ (from Nei and Tajima 1983). The two estimates are 113,000 (from $\pi = 0.60$) and 110,000 yrs B.P. (from $\delta = 0.44$) for divergence times between clones **AABBB** and **AAAAA**.

Another interesting aspect of the pattern of diversity are the relative geographical locations of clones **AAAAA** and **BBBBB**. Clone **BBBBB** in the southern area of British Columbia and clone **AAAAA** in British Columbia and Alberta are the most divergent pair of clones within Columbian ground squirrels inspite of their geographical proximity. It seems unlikely that there is any geological barrier between the Bluet and Moyie Lake sites since Columbian ground squirrels have been seen on the passes of Nelson Range (P. Marino, pers. comm.). Estimates of time of divergence between clones **AAAAA** and **BBBBB** are 164,000 (from $\pi = 0.87$) and 196,000 (from $\delta = 0.78$). Yet these clones do not overlap in their distribution. This is surprizing even in view of the low dispersal rate of females.

Over all, based on similarity and irrespective of geographical distribution, clone **AAAAA** is relatively distinct from the other three clones, which differ from each other by only one or two restriction site changes. Therefore, the Columbian ground squirrels apparently comprise two distinct subgroups. Because the rate of evolution of mtDNA has been demonstrated to be extremely rapid (Brown *et al.* 1979), this pattern of variation in the northern extent of the species range suggests that colonies are founded by a few females, and that there is limited dispersal by females between groups of colonies.

C. Geographical history of S. columbianus

The recent colonization of the northern extent of the Columbian ground squirrels range is unsurprizing in light of the geological history of the area. Until ca. 10,000 yrs. B.P. much of the sampled range of this species was covered by the Cordilleran and Laurentide glaciers. An ice free corridor between the two major glaciers existed along the eastern foothills of the Rocky Mountains as far north as the Jasper - Hinton area for 100,000 yrs B.P. (Rutter 1980). The Laurentide glacier had retreated from northern Alberta by ca. 10,000 yrs B.P. (Rutter 1977), and the Cordilleran glacier from the western foothills of the Rocky Mountains by ca. 11,000 yrs B.P. (Mayewski *et al.* 1981).

It is obvious that the estimated time of divergences between clones **AAAAA** and **AABBB** agree well with the estimated date of the ice free corridor. The estimated time of divergence for clones **AABBB** and **AAAAA** indicates that these clones may have existed for more than 100,000 yrs B.P. in the eastern ranges of the Rocky Mountains. Both biological and geological evidence exists for refugia in Mountain Park, Nordegg, and the Willmore Wilderness Provincial Park, Alta. (Packer and Vitt 1974; Pike 1980). I hypothesize that Columbian ground squirrel populations also survived the Wisconsinan ice age in refugia on the eastern ranges of the Rocky Mountains or in the ice free corridor to account for the amount of divergence between the colonies existing there.

In contrast, the western ranges, where colonies of Columbian ground squirrels are much more homogeneous, were probably colonized after the retreat of the Cordillerian glacier. However, the direction of dispersal may not have been from the southern range of the species as one would initially expect. The rooted matriarchal transformation series of Columbian ground squirrels indicates the direction of evolution of the four clones to be **BBBBB←ABBBB←→AABBB→AAAAA** . Thus the locations of the anscestral clone(s) are in the northern area of the species'

range. Following the direction of evolution within the transformation series, Columbian ground squirrels dispersed from the north to the south along the eastern ranges of the Rocky Mountains (Fig. 6). Along the western side of the Rocky Mountains, the direction of dispersal is ambiguous because the primitive clone occurs to the most southern extent of the sampled area. Therefore, dispersal could have occurred from north to south or from south to north on the western side of the Rocky Mountains. However, assuming no geological barrier prevents dispersal between the colonies at Bluet and Moyie Lake, I propose that **BBBBB** is not observed in Alberta and **AAAAA** is not observed farther west in British Columbia, because there has been insufficient time for these clones to disperse east and west. Under these assumptions, I hypothesize that dispersal on the western side of the Rocky Mountains also occurred from the north to the south after the retreat to the Cordilleran glacier.

The hypotheses proposed from these results may be further tested by including other related species, specifically *S. undulatus* which is reported to be the species most closely related to Columbian and Arctic ground squirrels (Robinson and Hoffmann 1975; Nadler and Hoffmann 1977; Nadler *et al.* 1984). Comparing restriction patterns of *S. undulatus* to those of Columbian ground squirrels may identify the root of the linear transformation series with greater confidence. Also, to test the linearity of the transformation series within Columbian ground squirrels, it would be necessary to examine individuals from colonies located between Rock Lake and Ya Ha Tinda Ranch, Alta., as well as from colonies in the United States. Whatever observations may occur in these future studies, a combination of recent colonization and low dispersal between established colonies could account for the present distribution of variation in sequence of mtDNA observed in this study.

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Table 1: Collection sites of Columbian ground squirrels trapped throughout Alberta and British Columbia

Code	Site Name	Location	Elevation (m)	No. of individuals
BT	Bluet, B.C.	49°28'N 117°23'W	750	3
WF	Wapiti Farms, Alta.	54°53'N 119°30'N	760	4
WM	WM Community Pasture, B.C.	54°10'N 122°36'W	610	4
BB	Big Berland River, Alta.	53°45'N 118°21'W	1370	1
TJ	Tete Jaune Cache, B.C.	53°N 119°30'W	915	4
VN	Vernon, B.C.	50°15'N 119°16'W	460	5
RL	Rock Lake, Alta.	53°29'N 118°15'W	1370	4
YR	Ya Ha Tinda Ranch, Alta.	51°44'N 115°32'W	1680	5
GD	Golden, B.C.	51°30'N 117°10'W	915	5
LG	Longview, Alta.	50°32'N 114°25'W	1220	24
ML	Moyie Lake, B.C.	49°22'N 115°50'W	1070	5
WT	Waterton, Alta.	49°14'N 113°55'W	1400	7
			Total	71

Table 2: Distribution of mtDNA clones among Columbian ground squirrels in Alberta and British Columbia

Location	Number of individuals	Composite mtDNA genotype[1]
BT	2	BBBBB
	1	ABBBB
WF	3	ABBBB
	1	A?BBB[2]
WM	3	ABBBB
	1	A?BBB
BB	1	ABBBB
TJ	4	ABBBB
VN	5	ABBBB
RL	3	AABBB
	1	A?BBB
YR	5	AAAAA
GD	4	AAAAA
	1	?AAAA
LG	16(21)[3]	AAAAA
	2	A?AAA
	1	AAAA?
ML	5	AAAAA
WT	3	AAAAA
	3	A?AAA
	1	AAAA?

[1]Each letter represents a restriction pattern of a particular endonuclease in order: Cfo I, Dde I, Eco RI, Hae III, Mbo I.
[2]? denotes for which endonuclease the restriction pattern could not be discerned.
[3]Five individuals analysed were juveniles of one litter and known to be clones of the dam, therefore these five are not independent samples.

Table 3: Number of restriction sites shared among Columbian, Arctic, and Richardson's ground squirrels. The diagonal contains the total number of mapped restriction sites and the off-diagonals contain the number of shared restriction sites of tetrameric endonucleases (upper line) and hexameric endonucleases (lower line) for each pairwise comparison.

Columbian ground squirrels			Arctic ground squirrels	Richardson's ground squirrels		
AAAAA	**AABBB and ABBBB**	**BBBBB**		**A**	**B**	
3	3	3	2	2	2	**AAAAA**
27	26	26	19	19	19	
	3	3	2	2	2	**AABBB and ABBBB**
	27	27	20	19	19	
		4	2	2	2	**BBBBB**
		27	20	19	19	
			4	4	4	Arctic ground squirrel
			26	22	22	
				4	4	Richardson's ground squirrels **A**
				25	24	
					4	**B**
					26	

Table 4: Maximum likelihood estimates of percent divergence (π) among Columbian, Arctic, and Richardson's ground squirrels as calculated from Nei and Tajima (1983).

Columbian ground squirrels		Arctic ground squirrels	Richardson's ground squirrels		
AABBB and ABBBB	**BBBBB**		**A**	**B**	
0.60	0.87	6.02	5.75	6.02	**AAAAA**
	0.29	5.26	5.75	6.02	**AABBB and ABBBB**
		5.56	6.06	6.32	**BBBBB**
			2.18	2.46	Arctic ground squirrels
				0.30	Richardson's ground squirrels **A**

Table 5: The number of restriction fragments shared among Columbian, Arctic, and Richardson's ground squirrels. The diagonal contains the total number of restriction fragments and the off-diagonals contain the number of shared fragments derived from digestion with tetrameric endonucleases (upper line) and hexameric endonucleases (lower line) for each pairwise comparison.

Columbian ground squirrels				Arctic ground squirrels	Richardson's ground squirrels		
AAAAA	**AABBB**	**ABBBB**	**BBBBB**		**A**	**B**	
49(14)[1]	42(14)	42(13)	41(13)	16	15	15	**AAAAA**
27	24	24	24	12	12	12	
	46(14)	16(13)	45(13)	15	14	14	**AABBB**
	27	27	27	13	12	12	
		46(15)	45(15)	15	14	14	**ABBBB**
		30	28	13	12	12	
			47(15)	15	14	14	**BBBBB**
			27	13	12	12	
				37	27	27	Arctic ground squirrels
				26	16	15	
					40	40	Richardson's ground squirrels **A**
					25	24	
						40	**B**
						26	

[1]Brackets contain the number of fragments derived from digestion with Dde I which were excluded from the interspecific comparisons.

Table 6: The average estimates of percent divergence (δ) among Columbian, Arctic, and Richardson's ground squirrels, as calculated from Nei and Li (1979).

Columbian ground squirrels			Arctic ground squirrels	Richardson's ground squirrels		
AABBB	**ABBBB**	**BBBBB**		**A**	**B**	
0.44	0.69	0.78	6.76	7.17	7.23	**AAAAA**
	0.24	0.34	6.65	7.34	7.40	**AABBB**
		0.09	6.65	7.34	7.40	**ABBBB**
			6.71	7.39	7.45	**BBBBB**
				2.86	3.11	Arctic ground squirrels
					0.17	Richardson's ground squirrels **A**

Figure 1: Locations of collection sites of Columbian ground squirrels in Alberta and British Columbia. Inset map shows location of collection of Arctic ground squirrels (A) and Richardson's ground squirrels (R), and the distribution of Columbian ground squirrels in Canada (stipled area). The number of different restriction patterns between two pairs of genotypes are illustrated by dashes across lines connecting most closely related clones. The dashes do not indicate the number of substitutions between the mtDNA sequences because for most of the restriction patterns, the number of sites lost or gained between variants could not be determined. Thus the three dashes across the line connecting AAAAA and AABBB indicate that these clones differ in three restriction patterns, specifically those of Eco RI, Hae III, and Mbo I.

BB = Big Berland River; BT = Bluet; GD = Golden; LG = Longview; ML = Moyie Lake; RL = Rock Lake; TJ = Tete Jaune Cache; VN = Vernon; WF = Wapiti Farms; WM = WM Community Pasture; WT = Waterton; YR = Ya Ha Tinda Ranch.

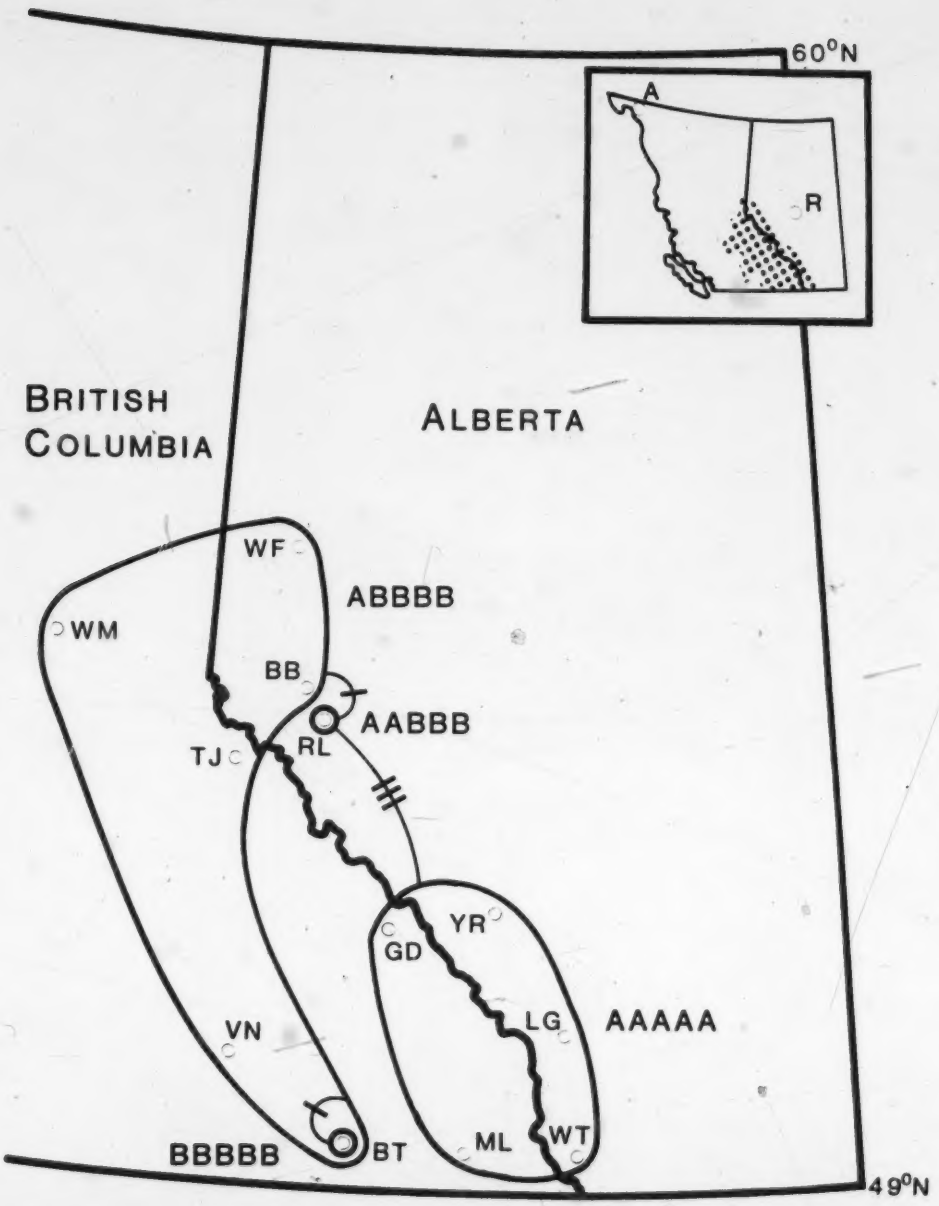

60°N
A
R
BRITISH
COLUMBIA
ALBERTA
WF
ABBBB
WM
BB
AABBB
TJ
RL
YR
GD
LG
AAAAA
VN
BBBBB
BT
ML
WT
49°N

Figure 2: Restriction patterns of the endonucleases, Hae III, Mbo I, Cfo I, and Eco RI in Columbian ground squirrels.

A1: The two variant restriction patterns derived from digestion with Hae III.

A2: The two variant restriction patterns derived from digestion with Mbo I.

B1: The two variant restriction patterns derived from digestion with Cfo I.

B2: The two variant restriction patterns derived from digestion with Eco RI.

The columns of numbers indicate in base pairs the length of the fragments of DNA.

The molecular weight standards are phage λ digested with Hind III and phage λ digested with Hind III and Eco RI run in one lane.

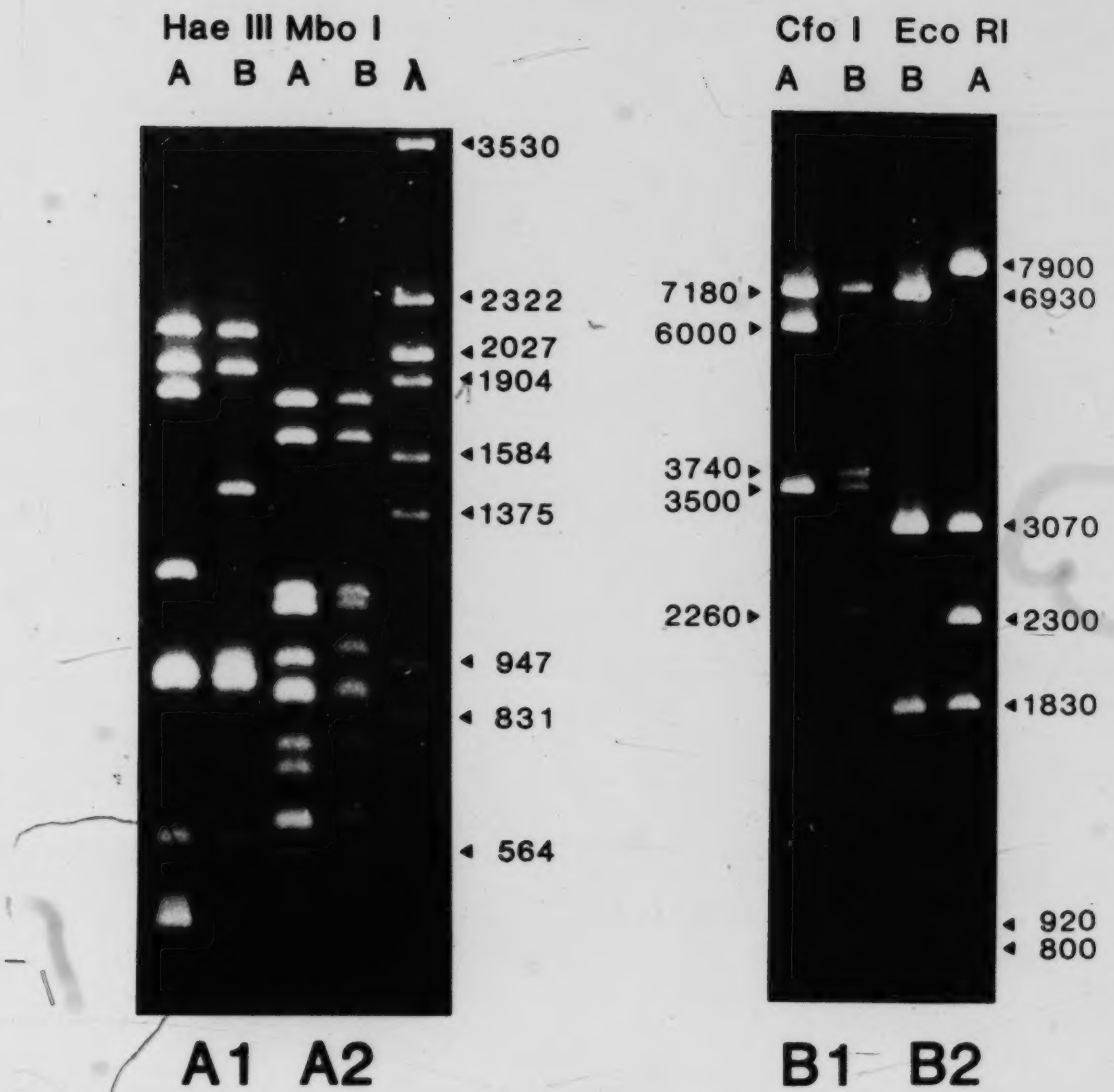
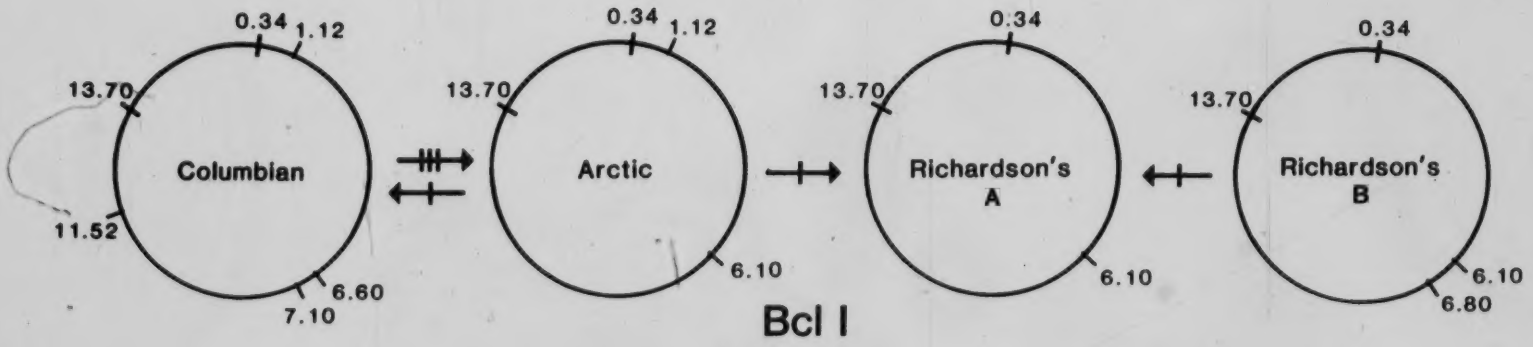
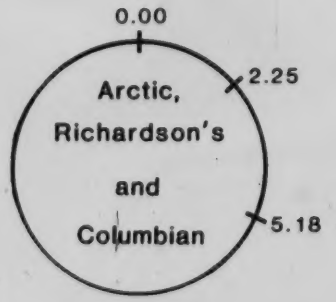

Hae III Mbo I
A B A B λ
3530
2322
2027
1904
1584
1375
947
831
564
A1 A2
Cfo I Eco RI
A B B A
7180
6000
3740
3500
2260
7900
6930
3070
2300
1830
920
800
B1 B2

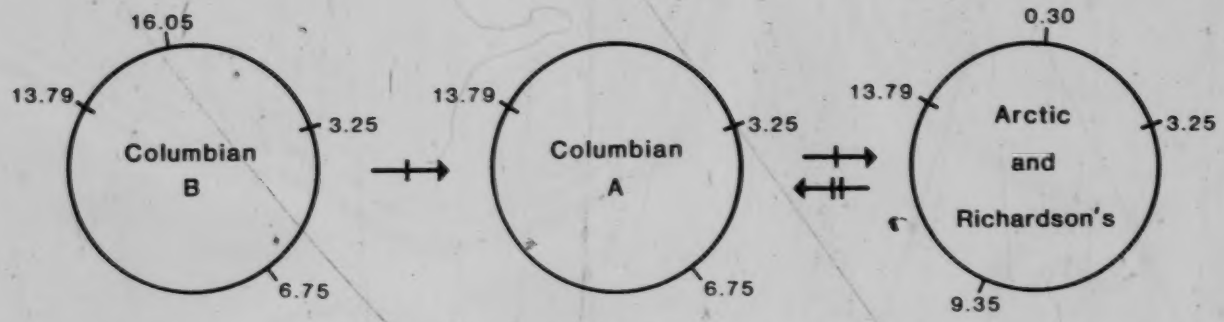
Figure 3: Restriction maps and transformation series of the three species of ground squirrels for Bcl I, Bgl II, Cfo I, Eco RI, Hind III, and Xba I. Lines spaning the circumference of the circle are interspecifically invariant sites; lines on the outside only of the circle are interspecifically variant sites. Site 0.0 is an arbitrarily chosen invariant site of Bgl II used as a reference point. All other sites of Bcl I, Bgl II, Cfo I, Eco RI, Hind III, and Xba I are indicated in kb from 0.0 on the mtDNA molecule, ca. 16600 bp total in length. The direction of the arrows indicates the direction of the loss of a restriction site.


0.34
1.12
13.70
Columbian
11.52
6.60
7.10
0.34
1.12
13.70
Arctic
6.10
0.34
13.70
Richardson's
A
6.10
0.34
13.70
Richardson's
B
6.10
6.80

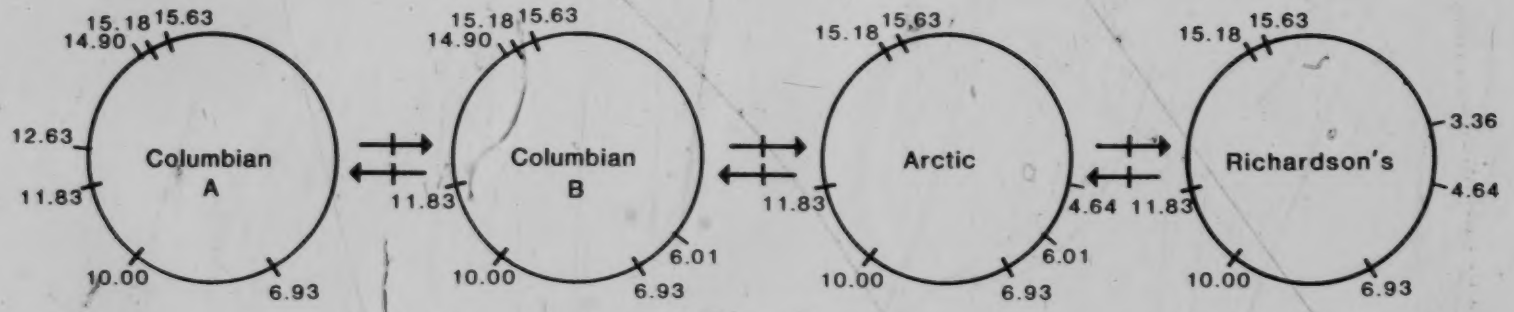
Bcl I


0.00
2.25
Arctic,
Richardson's
and
Columbian
5.18

Bgl II

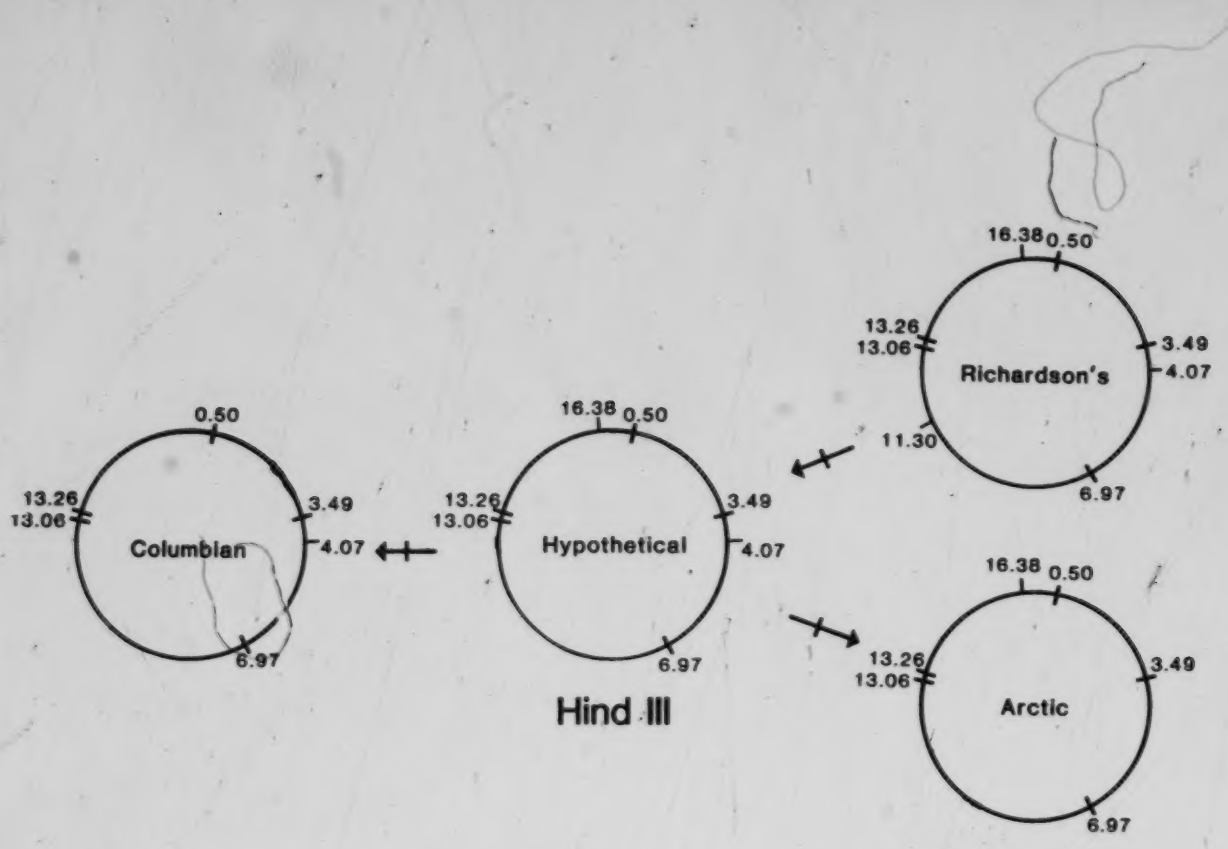

16.05
13.79
Columbian
B
3.25
6.75
13.79
Columbian
A
3.25
6.75
0.30
13.79
Arctic
and
Richardson's
3.25
9.35

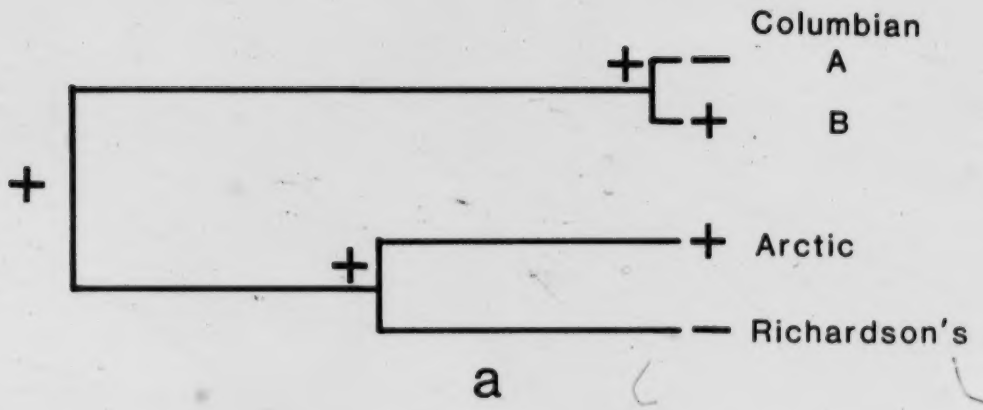
Cfo I


15.18
15.63
14.90
12.63
Columbian
A
11.83
10.00
6.93
11.83
15.18
15.63
14.90
Columbian
B
10.00
6.93
6.01
11.83
15.18
15.63
Arctic
11.83
10.00
6.93
6.01
4.64
11.83
15.18
15.63
Richardson's
3.36
4.64
10.00
6.93

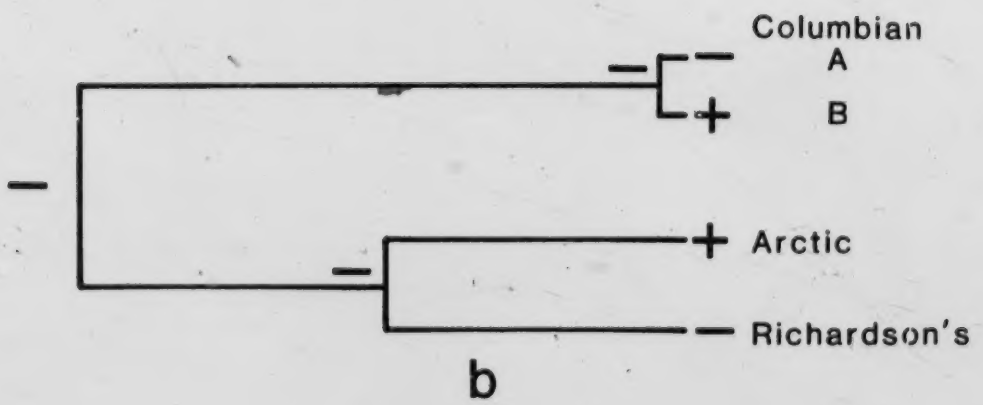
Eco RI

Figure 4: Alternative hypotheses for the character state of the presence (a) or absence (b) of the 6.01 kb Eco RI restriction site. The transformation series were rooted from estimated divergences of Columbian, Arctic, and Richardson's ground squirrels.


16.38
0.50
13.26
13.06
3.49
4.07
Richardson's
11.30
6.97
0.50
13.26
13.06
3.49
4.07
Columbian
6.97
16.38
0.50
13.26
13.06
3.49
4.07
Hypothetical
6.97
16.38
0.50
13.26
13.06
3.49
Arctic
6.97
Hind III
15.44
13.69
2.94
Columbian
12.19
10.84
15.18
15.44
13.69
Richardson's
12.19
15.18
15.44
13.69
Arctic
4.08
12.19
5.12
Xba I


Columbian
A
B
Arctic
Richardson's

a


Columbian
A
B
Arctic
Richardson's

b

Figure 5: Transformation series of Columbian, Arctic, and Richardson's ground squirrels based on all mapped restriction sites. Arrows indicate the direction of evolution; dashes across the arrows indicate the number of losses and/or gains of restriction sites. The double-headed arrow between Columbian ground squirrels and the hypothetical intermediate indicates that this transformation series is probably rooted between these two groups.

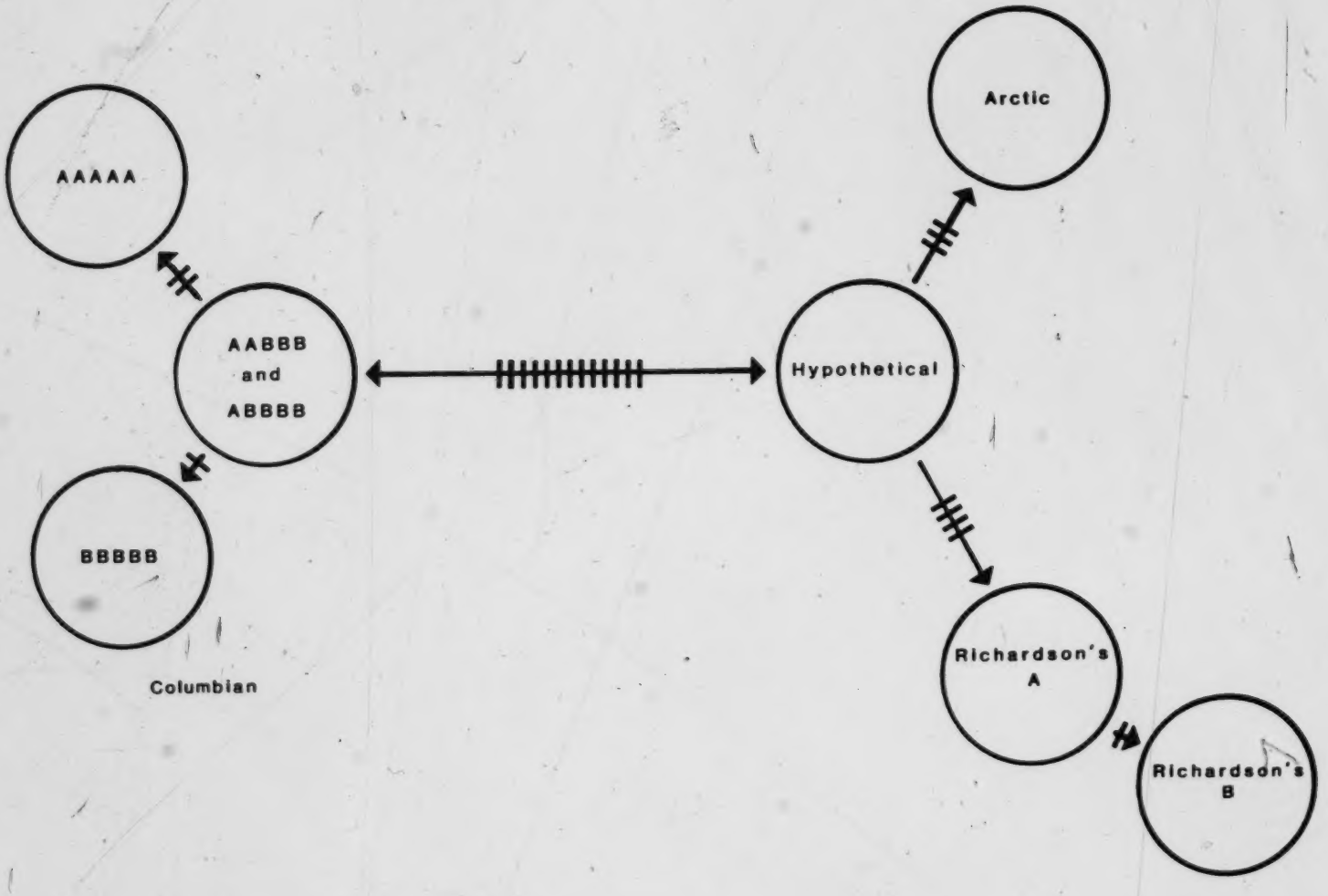

AAAAA
AABBB
and
ABBBB
BBBBB
Columbian
Hypothetical
Arctic
Richardson's
A
Richardson's
B

Figure 6: Possible dispersal routes of Columbian ground squirrels following the retreat of the Wisconcinan ice age. The arrows indicate the direction of dispersal.

BRITISH
COLUMBIA
ALBERTA

Appendix A: Materials and Methods

A. Field Procedures

Collection of Columbian ground squirrels

Eighty Columbian ground squirrels were collected live in 6 locations in Alberta and in 6 locations in British Columbia. A list of the number of squirrels trapped at each location is given in Table 1.

All Columbian ground squirrels used in this study were trapped in National Live traps. The traps were baited with peanut butter and set near burrow holes throught out the colony. After approximately two hours, any traps containing animals were fitted with a coarse mesh bag over the trap entrance. The entrance was opened and the squirrel forced to run into the mesh bag. Fish fingerling tags were clipped into one or both ears so that each squirrel could be identified by a unique number. Squirrels were sexed and weighed before they were transferred into a plastic cage with a metal grill lid. The cages were those used by the Animal Services of the Univ. of Alberta to house small mammals and measured 20 cm by 44 cm by 22 cm. In captivity, the squirrels were fed lab chow and sunflower seeds, also provided by Animal Services, and, when not travelling, the squirrels were given access to water bottles. Apple slices were also given to the squirrels as an additional food and water source. As soon as possible, captive squirrels were transported to the university in the back of a pickup truck whose cover was ventilated through an air vent. While at the university, the squirrels were cared for by Animal Services until required for mtDNA isolation.

Collection of Richardsons ground squirrels and Arctic ground squirrels

Liver from Richardson's ground squirrels, which were trapped near Edmonton, Alta. by either Mr. Paul Young, Mr. Micheal Jourdan, or Mr. Darrell Belke, was graciously supplied by Dr. Lawrence Wang of the University of Alberta. Arctic ground squirrels, collected from Haines Junction in Chillkat Pass, British Columbia, were generously supplied by Mr. James Schieck . The Arctic ground squirrels were flown to Edmonton, then transported by truck to the university. Treatment of the animals after arrival to the University of Alberta was identical to that given to Columbian ground squirrels.

B. Laboratory Procedures

Method for isolation of mitochondrial DNA from liver tissue

This method is slightly modified from that in Pedersen *et al.* (1978) and Wills *et al.* (1984). All steps are performed at 4°C unless otherwise stated. All solutions are listed in Table A1.

1. Liver (and occasionally kidney) tissue was removed from recently decapitated ground squirrels and immediately transferred into Homogenizing-medium (H-medium).
2. At most 10 g of tissue was minced, then ground into an homogenate with a teflon-glass tissue grinder. A total of 20 ml of H-medium was added to the homogenate, and equally divided into two 50 ml centrifuge tubes.
3. The homogenate was centrifuged at 1085 g's (in a Sorvall SS-34 rotor at 3000 rpm) for 3 min; the volume of the supernatant of mitochondria and lighter particles was measured and separated from the pellet and kept on ice for later centrifugation.
4. The pellet of heavier cellular debris and whole cells was resuspended in a volume of H-medium equal to that of the supernatant removed in step 3.
5. Steps 3. and 4. were repeated with the resuspension two times. The pellet from

the third spin was discarded. The resulting three supernatants were combined and spun at 6780 g's (Sorvall SS-34, 7500 rpm) for 15 min.

6. The supernatant of light particles and proteins was discarded and the pellet of mitochondria was resuspended in 1/2 the volume of combined supernatants obtained by step 5, then the suspension was spun at 20200 g's (Sorvall SS-34, 13000 rpm) for 10 min.

7. The pellet was again resuspended in 1/4 the volume obtained in step 5. with H-medium. $MgCl_2$ and DNase were added to the resuspension to final concentrations of 0.01 M and 0.05 mg/ml, respectively. This suspension was incubated on ice for 1/2 to 1 hr, to reduce any nuclear DNA, which was not contained in the nuclei, to nucleotides, then the suspension was spun at 20200 g's (Sorvall SS-34, 13000 rpm) for 15 min.

8. The pellet was resuspended in 2 ml lysing medium and Pronase (Type XIV) was added to a final concentration of 0.4 mg/ml and incubated overnight at 37°C to inactivate DNase added in step 7. and any native nucleases or other proteins.

9. Dodecyl sodium sulfate (SDS) was added to the suspension to a final concentration of 1% or 2%, and incubated for 1 to several hrs on ice, to disrupt cellular membranes.

10. The SDS and membrane lipids were removed by 1 to 3 phenol extractions, and phenol was removed from the aqueous mtDNA solution by extraction with a chloroform and isoamyl alcohol mixture (24:1 by volume).

11. MtDNA was recovered with ethanol precipitation at -20°C by addition of sodium acetate and ethanol to final concentrations of 0.1 M and approximately 30%, respectively.

12. Ethanol was removed from mtDNA by spinning precipitated DNA at 20200 g's for 30 min. and dessicating the resulting pellet under vacuum.

13. The mtDNA was resuspended in 1 ml TE buffer and stored at -20°C.

14. For most samples it was necessary to reduce the amount of RNA contamination, since RNA inhibits the activity of restriction endonucleases and makes analysis of the restriction pattern difficult. This was done by first incubating the sample at 65°C to inactivate any persistant nucleases, and then, by adding RNase to a final concentration of 0.4 mg/ml and incubating at 50°C for 1 hr.

15. Even after the above treatment, some samples could not be digested with a few of the ten endonucleases. In this event, any impurities, such as proteins or polysaccharides, present in the sample were removed by NACS chromatography as specified by Bethesda Research Laboratories. These samples were prepared for purification on a NACS column by one phenol extraction, one chloroform-isoamyl alcohol extraction, and ethanol precipitaion as previously described in steps 12 to 14.

16. In most cases, the above method of purification was sufficient to purify samples enough to be digested by all ten endonucleases, however, if this were not the case those samples were precipitated by a method described in Hoopes and McClure (1981).

Restriction analysis

MtDNA from individuals was digested with ten endonucleases, which recognize the nucleotide sequence in parentheses: Bcl I (TGATCA), Bgl II (AGATCT), Cfo I (GCGC), Dde I (CTNAG), Eco RI (GAATTC), Hae III (GGCC), Hind III (AAGCTT), Hpa II and/or Msp I (CCGG), Mbo I (GATC), and Xba I (TCTAGA). MtDNA from Arctic and Richardson's ground squirrel were not digested with Dde I for lack of sample. Other endonucleases were also tried: Alu I, Bam HI, Eco RV, Hinf I, Kpn I, Pst I, Rsa I, Sma I, Sst I, Tqu I, Rsa I, and Xho I, which were unsuitable for one of the following reasons: (1) no site was present, (2) impurities, which could not be eliminated, prevented restriction, and (3) the fragments produced were too small to be resolved on agarose. The

appropriate conditions for restriction with each endonuclease are listed in Table A2, and follow the suggestions of Bethesda Research Laboratories, from which the endonucleases were obtained, or Maniatis *et al.* (1982). Fragments were separated by gel electrophoresis on agarose gels of appropriate concentrations (listed in Table A1) and sized by comparison to mobilities of fragments of known lengths; fragments used as standards were obtained by restriction of phage λ DNA with Hind III, both Bam HI and Eco RI, and both Hind III and Eco RI. Fragments were visualized by staining with ethidium bromide and photographed under UV light.

Construction of Restriction Maps

A mtDNA restriction map of six endonucleases, Bcl I, Bgl II, Cfo I, Eco RI, Hind III, and Xba I, was constructed by examining fragment patterns from partial and double digests. Partial digests were achieved by reducing the length of the incubation period at 37°C; complete digests require 1 to 3 hrs for completion. If the sample is incubated with endonuclease for only 10 to 30 min, the endonuclease does not digest all sites and since the process is random, the DNA molecules will be digested at only one or more of the restriction sites. The larger fragments from a partial digest contain restriction sites which have not been cleaved. Therefore, to every combination of two, three, etc. adjacent fragments there should correspond a large fragment. The adjacent fragments can be deduced by summing the lengths of fragments from the complete digest to equal the larger fragments of the partial digest. In double digests the DNA molecules are digested with two endonucleases. If the two endonucleases require different incubation conditions, then the DNA is digested sequentially, first under conditions favorable to one endonuclease, then under conditions favorable to the second. This produces many fragments of lengths smaller than those of either single digest because fragments from one endonuclease restriction contain the restriction sites of other endonucleases.

The spatial arrangement of restriction sites of one endonuclease to restriction sites of the second can be deduced by superimposing the restriction maps of each restriction endonuclease (gained from the partial digests). The correct orientation results in fragments of the correct lengths. This analysis was perfomed for Bcl I, Bgl II, Cfo I, Eco RI, Hind III, and Xba I to map these sites with respect to the invariant restriction sites of Bgl II.

C. Restriction Patterns

This section is a tabulation of fragment lengths derived from restriction with the ten endonucleases used in this study (Table A3). Also included are those fragments which appear only in partial digests (PARTIAL FRAGMENTS), and those fragments derived from digestion of the two indicated endonucleases.

Literature Cited

Hoopes, B.C. and W.R. McClure. 1981. Studies on the selectivity of DNA precipitation by spermine. Nucl. Acids Res. 9:5493-5504.

Maniatis, T., E.F. Fritsch, and J. Sambrook. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Pedersen, P.L., J.W. Greenwalt, B. Reynafarje, J. Hullihen, G.L. Decker, J.W. Soper, and E. Bustaments. 1978. Preparation and characterization of mitochondria and submitochondrial particles of rat liver and liver-derived tissues. *In* Methods in Cell Biology. Vol. X. *Edited by* D.M. Prescott.

Wills, J.W., B.A. Casker, K. Sirotkin, and W.S. Riggsby. 1984. Repetitive DNA of *Candida albians*: nuclear and mitochaondrial components. J. Bacter. 157:918-924.

Table A1: Solutions required for isolation and digestion of mtDNA.

Solution	Concentrations of compounds	pH	Reference
Homogenizing medium	0.07 M Sucrose 0.21 M Mannitol 1.9 mM HEPES 0.5% BSA	8.5	Pedersen *et al.* (1978)
Lysing medium	0.10 M EDTA 0.10 M Tris 0.20 M NaCl	to 7.5 with HCl	Wills *et al.* (1984)
TE	10 mM Tris 1 mM EDTA	to 7.4 with HCl	Maniatis *et al.* (1982)
Core restriction buffer	50 mM NaCl 50 mM Tris 10 mM $MgCl_2$	8.0	as recommended by the manufacturer Bethesda Research Labs. (BRL)
Low salt restriction buffer	10 mM Tris 10 mM $MgCl_2$ 1 mM DTT	7.5	Maniatis *et al.* (1982)
Medium salt restriction buffer	50 mM NaCl 10 mM Tris 10 mM $MgCl_2$ 1 mM DTT	7.5	Maniatis *et al.* (1982)
High salt restriction buffer	100 mM NaCl 50 mM Tris 10 mM $MgCl_2$ 1 mM DDT	7.5	Maniatis *et al.* (1982)
Xba I restriction buffer	100 mM NaCl 6 mM Tris 6 mM $MgCl_2$	7.4	BRL

Abbreviations used in Table A1: BSA = Bovine albumin, DTT = Dithiothreitol, EDTA = ethylenediaminetetraacetic acid, HEPES = N-2-HydroxyethylpiperazineN'-2-ethanesulfonic acid, Tris = Tris(hydroxymethyl)aminomethane.

Table A2: Digest and electrophoresis conditions

Endonuclease	Restriction buffer	Time (hr)	Agarose concentration (%)
Bcl I	Core	3	1.5
Bgl II	Core	1	0.7
Cfo I	Low salt	1	0.7
Dde I	Core	3	2.0
Eco RI	Medium salt	2	0.9
Hae III	Core	2	2.0 - 1.5
Hind III	Core	2	0.9
Hpa II	Low salt	2	2.0 - 1.5
Mbo I	Core	3	2.0 - 1.5
Xba I	Xba I	3	1.0

All restrictions were at 37°C, except with Bcl I which were at 50°C, and a voltage of 2 v/cm was applied to all agarose gels for 12 to 18 hrs.

Table A3: Fragment lengths derived from restrictions with each of ten endonucleases for three species of ground squirrels. Partial fragments are those fragments which appear only in partial digests. Two endonucleases listed in the second column indicate a double digestion.

Species	Endonuclease	Pattern	Fragment lengths (kb)
columbianus	Bcl I		5.48; 4.56; 3.12; 2.20; 0.78; 0.50 PARTIAL FRAGMENTS 3.98; 5.30; 5.94; 6.10; 6.35; 6.46
parryii	Bcl I		7.50; 4.89; 3.12; 0.78 PARTIAL FRAGMENTS 3.95; 5.84; 8.15; 9.76; 11.14; 11.55; 12.79
richardsonii	Bcl I	A	7.50; 5.78; 3.12 PARTIAL FRAGMENTS 9.08; 11.51; 14.67
richardsonii	Bcl I	B	6.90; 5.78; 3.12; 0.70 PARTIAL FRAGMENTS 6.24; 6.86; 9.08; 11.51; 14.67
columbianus *parryii* *richardsonii*	Bgl II		11.80; 2.93; 2.25 PARTIAL FRAGMENTS 5.20; 14.76
columbianus	Cfo I	A	7.01; 6.03; 3.54 PARTIAL FRAGMENTS 9.41; 10.62; 12.91
columbianus	Cfo I	B	7.01; 3.74; 3.54; 2.26
richardsonii *parryii*	Cfo I		6.10; 4.40; 3.05; 2.95 PARTIAL FRAGMENTS 7.51; 9.09; 10.62; 10.49; 13.64; 15.81
columbianus	Dde I	A	1.13; 0.92; 0.82; 0.79; 0.73; 0.70; 0.69; 0.67; 0.66; 0.65; 0.63; 0.60; 0.59; 0.58; 0.57
columbianus	Dde I	B	1.49; 1.13; 0.82; 0.79; 0.73; 0.69; 0.67; 0.66; 0.65; 0.63; 0.60; 0.59; 0.58; 0.57
columbianus	Eco RI	A	6.93; 3.07; 3.07; 1.83; 0.92; 0.45; 0.28 PARTIAL FRAGMENTS 3.35; 3.37; 3.90; 4.90; 5.10; 5.40; 5.67; 5.89; 7.34; 7.81; 8.64; 11.79; 12.42; 15.75

columbianus	Eco RI	B	7.85; 3.07; 2.30; 1.83; 0.80; 0.45; 0.28 PARTIAL FRAGMENTS 2.50; 3.35; 3.73; 4.90; 5.10; 5.89; 8.58; 13.75
parryii	Eco RI		5.55; 3.35; 3.07; 1.83; 1.36; 0.92; 0.45 PARTIAL FRAGMENTS 2.52; 3.89; 4.89; 5.90; 7.54; 8.62; 9.72; 11.35
richardsonii	Eco RI		4.25; 3.35; 3.07; 2.29; 1.83; 1.28; 0.45 PARTIAL FRAGMENTS 3.42; 3.74; 4.59; 4.78; 5.65; 6.09; 8.85
columbianus	Hae III	A	2.12; 1.93; 1.82; 1.16; 0.91; 0.91; 0.90; 0.65; 0.55; 0.52; 0.52; 0.48; 0.47; 0.40; 0.38; 0.31; 0.30; 0.28
columbianus	Hae III	B	2.12; 1.93; 1.46; 0.93; 0.91; 0.91; 0.90; 0.65; 0.52; 0.52; 0.48; 0.47; 0.40; 0.38; 0.31; 0.30; 0.28
parryii	Hae III		1.90; 1.23; 1.02; 0.93; 0.85; 0.82; 0.75; 0.65
richardsonii	Hae III		1.83; 1.46; 1.23; 1.02; 1.00; 0.93; 0.84; 0.76; 0.73; 0.65; 0.63; 0.61
columbianus	Hind III		6.06; 3.90; 2.90; 2.99; 0.58; 0.20 PARTIAL FRAGMENTS 3.29; 3.34; 3.90; 6.54; 7.05; 7.90; 9.83; 10.55; 11.37; 14.14
parryii	Hind III		6.06; 3.45; 3.12; 2.99; 0.80; 0.20
richardsonii	Hind III		4.33; 3.12; 2.99; 2.90; 1.76; 0.80; 0.58; 0.20 PARTIAL FRAGMENTS 1.84; 3.19; 3.29; 3.34; 3.66; 3.77; 3.90; 4.33; 5.03; 5.96; 6.34; 6.47;6.61; 7.05; 7.72; 7.90; 8.50; 9.69; 10.25; 11.20; 11.92; 13.17; 13.64
columbianus	Mbo I	A	1.78; 1.63; 1.09; 1.06; 0.94; 0.87; 0.78; 0.73; 0.64; 0.59; 0.53; 0.49; 0.45; 0.42; 0.39; 0.30

columbianus	Mbo I	B	1.78; 1.63; 1.09; 1.06; 0.97; 0.86; 0.78; 0.65; 0.59; 0.49; 0.45; 0.42; 0.39; 0.30
parryii	Mbo I		2.11; 1.63; 1.09; 0.94; 0.87; 0.78; 0.75; 0.73; 0.68; 0.65; 0.59; 0.49; 0.45; 0.42; 0.39; 0.30
richardsonii	Mbo I		1.63; 1.43; 1.09; 1.06; 0.94; 0.87; 0.78; 0.74; 0.68; 0.65; 0.59; 0.49; 0.45; 0.42; 0.39; 0.30
columbianus	Msp I		2.41; 2.22; 2.03; 1.98; 1.77; 1.71; 1.45; 1.26; 1.10
parryii	Msp I		4.52; 3.80; 2.13; 1.98; 1.77; 1.59; 1.45; 1.10; 1.06
richardsonii	Msp I		4.52; 3.80; 2.13; 1.98; 1.77; 1.59; 1.45; 1.10
columbianus	Xba I		8.00; 4.09; 1.75; 1.49; 1.35 PARTIAL FRAGMENTS 2.68; 3.01; 4.45; 5.41;7 .21; 9.69; 11.03; 12.31; 13.64; 15.25; 16.56
parryii	Xba I		6.90; 5.24; 1.49; 1.49; 1.04; 0.26 PARTIAL FRAGMENTS 1.75; 2.92; 3.19; 5.62; 6.54; 6.90; 8.95; 9.43; 10.40; 16.53
richardsonii	Xba I		13.20; 1.49; 1.49; 0.26 PARTIAL FRAGMENTS 1.75; 2.92; 16.56
columbianus	Bgl II / Bcl I		4.56; 2.93; 2.83; 2.20; 1.43; 1.12; 0.78
richardsonii	Bgl II / Bcl I	B	6.90; 2.93; 2.83; 1.94
columbianus	Bgl II / Cfo I	A	7.01; 2.75; 2.25; 1.90; 1.54; 1.00
richardsonii	Bgl II / Cfo I		4.40; 2.75; 2.00; 1.90; 1.00
columbianus	Bgl II / Eco RI	A	3.07; 3.07; 2.80 2.25; 1.83; 0.92; 0.90; 0.45
columbianus	Bgl II / Eco RI	B	3.07; 2.80; 2.30; 2.25; 1.83; 1.75; 0.90
parryii	Bgl II / Eco RI		3.35; 3.07; 2.35; 2.25; 1.83; 0.90
richardsonii	Bgl II / Eco RI		3.35; 3.07; 2.25; 1.83; 1.75; 1.28; 1.15; 0.57

columbianus	Bgl II / Hind III		6.06; 3.39; 1.83; 1.73; 1.19; 1.16
richardsonii	Bgl II / Hind III		4.33; 3.12; 1.83; 1.73; 1.19; 1.16; 0.70
columbianus	Bgl II / Xba I		6.68; 4.09; 2.93; 2.90; 2.25
parryii	Bgl II / Xba I		6.68; 2.25; 1.74; 1.04; 1.49; 1.16
richardsonii	Bgl II / Xba I		6.68; 4.30; 4.10; 2.93; 2.25; 1.16
columbianus	Cfo I / Eco RI	A / A	4.10; 3.07; 2.72; 1.89; 1.83; 1.18; 0.74; 0.45
columbianus	Cfo I / Eco RI	A / B	4.10; 3.54; 3.07; 1.80; 1.18; 1.14; 0.83; 0.45
richardsonii	Cfo I / Eco RI		3.07; 2.40; 2.29; 1.89; 1.83; 1.50